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(54) Title: METHOD AND APPARATUS FOR SELECTIVE BIOLOGICAL MATERIAL DETECTION

(57) Abstract: The present invention relates to bioassay materials useful for the detection of toxic substances and, more particularly, to packaging materials for food and other products, along with methods for their manufacture and use. The invention provides a unique composite material capable of detecting and identifying multiple biological material within a single package. The biological material identification system is designed for incorporation into existing types of flexible packaging material such as polyvinylchloride or polyolefin films, and its introduction into the existing packaging infrastructure will require little or no change to present systems and procedures.



**Method and Apparatus for Selective Biological Material  
Detection**

**Reference to Related Applications:**

This application is a continuation-in-part of S.N.  
09/218,827, filed on Dec. 22, 1998 and now U.S. Patent  
6,051,388, having an issue date of April 18, 2000, the  
contents of which are herein incorporated by reference.

**Field of the Invention**

This invention relates to the detection of pathogenic  
microorganisms, or biological materials, and more  
particularly relates to a composite bioassay material  
useful for the detection of particular toxic substances,  
its method of manufacture and method of use, wherein the  
composite material is particularly useful for food  
packaging and the like, and is capable of simultaneously  
detecting and identifying a multiplicity of such  
biological materials.

**Background of the Invention**

Although considerable effort and expense have been  
put forth in an effort to control food borne pathogenic  
microorganisms, there nevertheless exist significant  
safety problems in the supply of packaged food. For  
example, numerous outbreaks of food poisoning brought  
about by foodstuffs contaminated with strains of the E-  
Coli, Campylobacter, Listeria, Cyclospora and Salmonella  
microorganisms have caused illness and even death, not to  
mention a tremendous loss of revenue for food producers.  
These and other microorganisms can inadvertently taint  
food, even when reasonably careful food handling  
procedures are followed. The possibility of accidental  
contamination, for example by temperature abuse, in and of  
itself, is enough to warrant incorporation of safe and



1 effective biological material diagnosis and detection  
2 procedures. Further complicating the situation is the  
3 very real possibility that a terrorist organization might  
4 target either the food or water supply of a municipality  
5 or even a nation itself, by attempting to include a  
6 pathogenic microorganism or toxic contaminant capable of  
7 causing widespread illness or even death. If, by accident  
8 or design, the food supply of a particular population were  
9 to be contaminated, it is not only imperative that the  
10 population be alerted to the contamination, but it is  
11 further necessary that the particular contaminant be  
12 quickly and precisely pinpointed so that appropriate  
13 countermeasures may be taken.

14 Thus, if it were possible to readily substitute  
15 standard packaging materials with a flexible material  
16 capable of  
17 1) quickly and easily detecting the presence, and  
18 2) indicating the particular identity of a variety of  
19 pathogenic biological materials, a long felt need would be  
20 satisfied.

21

#### 22 **Description of the Prior Art**

23 The Berkeley Lab Research News of 12/10/96, in an  
24 article entitle "New Sensor Provides First Instant Test  
25 for Toxic E.Coli Organism" reports on the work of Stevens  
26 and Cheng to develop sensors capable of detecting E. Coli  
27 strain 0157:H7. A color change from blue to red  
28 instantaneously signals the presence of the virulent E.  
29 Coli 0157:H7 microorganism. Prior art required test  
30 sampling and a 24 hour culture period in order to  
31 determine the presence of the E. Coli microorganism,  
32 requiring the use of a variety of diagnostic tools  
33 including dyes and microscopes. An alternative technique,  
34 involving the use of polymerase chain reaction technology,  
35 multiplies the amount of DNA present in a sample until it



1 reaches a detectable level. This test requires several  
2 hours before results can be obtained. The Berkeley sensor  
3 is inexpensive and may be placed on a variety of materials  
4 such as plastic, paper, or glass, e.g. within a bottle cap  
5 or container lid. Multiple copies of a single molecule  
6 are fabricated into a thin film which has a two part  
7 composite structure. The surface binds the biological  
8 material while the backbone underlying the surface is the  
9 color-changing signaling system.

10 The Berkeley researchers do not teach the concept of  
11 incorporating any means for self-detection within food  
12 packaging, nor do they contemplate the inclusion of  
13 multiple means capable of both detecting and identifying  
14 the source of pathogenic contamination to a technically  
15 untrained end user, e.g. the food purchaser or consumer.

16 Wang et al, in an article entitled "An immune-  
17 capturing and concentrating procedure for Escherichia coli  
18 0157:H7 and its detection by epifluorescence microscopy"  
19 published in Food Microbiology, 1998, Vol. 15 discloses  
20 the capture of E. coli on a polyvinylchloride sheet coated  
21 with polyclonal anti-E. coli 0157:H7 antibody and stained  
22 with fluorescein-labeled anti-E. coli 0157:H7. After  
23 being scraped from the PVC surface, the cells were  
24 subjected to epifluorescence microscopy for determining  
25 presence and concentration. The reference fails to teach  
26 or suggest the concept of incorporating any means for  
27 self-detection within food packaging, nor does it  
28 contemplate the inclusion of multiple means capable of  
29 both detecting and identifying the source of pathogenic  
30 contamination to a technically untrained end user, e.g.  
31 the food purchaser or consumer, and especially fails to  
32 disclose such detection without the use of specialized  
33 detection techniques and equipment.

34 U.S. Patent 5,776,672 discloses a single stranded  
35 nucleic acid probe having a base sequence complementary to



1 the gene to be detected which is immobilized onto the  
2 surface of an optical fiber and then reacted with the gene  
3 sample denatured to a single stranded form. The nucleic  
4 acid probe, hybridized with the gene is detected by  
5 electrochemical or optical detection methodology. In  
6 contrast to the instantly disclosed invention, this  
7 reference does not suggest the immobilization of the probe  
8 onto a flexible polyvinylchloride or polyolefin film, nor  
9 does it suggest the utilization of gelcoats having varying  
10 porosities to act as a control or limiting agent with  
11 respect to the migration of antibodies or microbial  
12 material through the bioassay test material, or to serve  
13 as a medium for enhancement of the growth of the microbial  
14 material.

15 U.S. Patent 5,756,291 discloses a method of  
16 identifying oligomer sequences. The method generates  
17 aptamers which are capable of binding to serum factors and  
18 all surface molecules. Complexation of the target  
19 molecules with a mixture of nucleotides occurs under  
20 conditions wherein a complex is formed with the specific  
21 binding sequences but not with the other members of the  
22 oligonucleotide mixture. The reference fails to suggest  
23 the immobilization of the aptamers upon a flexible  
24 polyvinylchloride or polyolefin base material, nor does it  
25 suggest the use of a protective gelcoat layer which acts  
26 as a means to selectively control the migration of  
27 antibodies and antigens, or to serve as a medium for  
28 enhancement of the growth of microbial material.

29

### 30 Summary of the Invention

31 The present invention relates to packaging materials  
32 for food and other products, along with methods for their  
33 manufacture and use. The presence of undesirable  
34 biological materials in the packaged material is readily  
35 ascertained by the consumer, merchant, regulator, etc.



1 under ordinary conditions and without the use of special  
2 equipment. A multiplicity of biological materials  
3 threaten our food supply. The present invention provides  
4 a unique composite material capable of detecting and  
5 identifying multiple biological materials within a single  
6 package. The biological material identification system is  
7 designed for incorporation into existing types of flexible  
8 packaging material such as polyvinylchloride and  
9 polyolefin films, and its introduction into the existing  
10 packaging infrastructure will require little or no change  
11 to present systems or procedures. Thus, the widespread  
12 inclusion of the biological material detecting system of  
13 the instant invention will be both efficient and  
14 economical.

15 In one embodiment of the invention the biological  
16 material detecting system prints a pattern containing  
17 several antibodies or aptamers, derived from plant or  
18 animal origins, onto a packaging material which is usually  
19 a type of polymeric film, preferably a polyvinylchloride  
20 or polyolefin film and most preferably a polyethylene film  
21 which has undergone a surface treatment, e.g. corona  
22 discharge to enhance the film's ability to immobilize the  
23 antibodies upon its surface. The agents are protected by  
24 a special abrasion resistant gel coat in which the  
25 porosity is tailored to control the ability of certain  
26 antibodies, toxic substances, etc. to migrate  
27 therethrough. Each antibody is specific to a particular  
28 biological material and is printed having a distinctive  
29 icon shape. The detection system may contain any number  
30 of antibodies capable of detecting a variety of common  
31 toxic food microbes; although any number of microbes may  
32 be identified via the inventive concept taught herein, for  
33 the purpose of this description, the microbes of interest  
34 will be limited to E.Coli, Salmonella, Listeria and  
35 Cyclospora.



1           An important feature of the biological material  
2     detection system is its all-encompassing presence around  
3     and upon the product being packaged. Since the biological  
4     material detecting system is designed as an integral part  
5     of 100% of the packaging material and covers all surfaces  
6     as utilized, there is no part of the packaged product  
7     which can be exposed to undetected microbes. In the past;  
8     the use of single location or *in situ* detectors have left  
9     a majority of the area around and upon the packaged  
10    product exposed to undetected microbes. This greatly  
11    increased the chance that a spoiled or tainted product  
12    might be inadvertently consumed before the toxic agent had  
13    spread to the location of the *in situ* detector. The  
14    biological material detection system of the present  
15    invention avoids this problem by providing a plurality of  
16    individual detectors per unit area which are effective to  
17    insure positive detection of any pathogenic microorganisms  
18    within the product being tested. In order to be effective  
19    a particular degree of sensitivity is required, e.g. the  
20    detecting system must be capable of positively identifying  
21    one microbial cell in a 25 gram meat sample. In a  
22    preferred embodiment, four detectors per square inch of  
23    packaging material surface have been utilized, and in a  
24    most preferred embodiment nine or more detectors per  
25    square inch are incorporated upon the film's surface.

26           By use of the biological material detection system of  
27    the present invention a packager or processor can  
28    independently determine the multiplicity and identity of  
29    those biological materials against which the packaged  
30    product is to be protected. Although it is envisioned  
31    that the large majority of biological material detection  
32    treated packaging will be generic to approximately four of  
33    the most common microbes, the system will nevertheless  
34    allow each user to customize the protection offered to the  
35    public.



1           The biological material detecting system will not  
2 merely detect the presence of biological materials, it  
3 will also identify the particular biological materials  
4 located in a packaged product. This unique feature allows  
5 for the immediate identification of each particular  
6 biological material present since the antibodies are  
7 specific to a detector having a definitive icon shape or  
8 other identifying characteristic. Although the end use  
9 consumer is primarily interested in whether a food product  
10 is, or is not, contaminated per se, the ability to detect  
11 and identify the particular biological material  
12 immediately is of immeasurable value to merchants,  
13 processors, regulators and health officials. The ability  
14 to immediately identify a toxic material will lead to  
15 greatly reduced response times to health threats that  
16 might be caused by the biological material and will also  
17 enhance the ability for authorities to locate the source  
18 of the problem. The biological material detecting system  
19 of the present invention exhibits an active shelf life in  
20 excess of 1 year under normal operating conditions. This  
21 enhances the use of a biological material detection system  
22 on products which are intended to be stored for long  
23 periods of time. If these products are stored so as to be  
24 ready for immediate use in some time of emergency, then it  
25 is extremely beneficial to definitely be able to determine  
26 the safety of the product at the time that it is to be  
27 used.

28           One particularly important feature of the biological  
29 material detecting system of the instant invention is its  
30 ability to quantitatively sensitize the reagents so as to  
31 visually identify only those biological materials which  
32 have reached a predetermined concentration or threshold  
33 level which is deemed to be harmful to humans.

34           For example, almost all poultry meat contain traces  
35 of the salmonella bacteria. In most cases, the salmonella



1 levels have not reached a harmful level of concentration.  
2 The biological material detecting reagents are designed to  
3 visually report only those instances where the level of  
4 concentration of biological materials are deemed harmful  
5 by health regulatory bodies.

6 The method of production of the biological material  
7 detecting system is designed to be easily incorporated  
8 within the packaging infrastructure of existing systems  
9 without disruption of the systems or the procedures under  
10 which they are operating. The biological material  
11 detecting system can be incorporated onto packaging films  
12 which are produced by the packager, or those which are  
13 supplied by a film manufacturer. The apparatus necessary  
14 for applying the biological material detecting system may  
15 be easily located at the beginning of any continuous  
16 process such as printing or laminating and will operate as  
17 an integral part of an existing system.

18 The biological material detecting system of the  
19 instant invention represents an entirely new packaging  
20 material which is designed to inform the consumer of the  
21 presence of certain biological materials or pathogens  
22 present in food stuffs or other materials packaged within  
23 the detecting system. The system is designed so that the  
24 presence of a biological material is presented to the  
25 consumer in a distinct, unmistakable manner which is  
26 easily visible to the naked eye.

27 Recent outbreaks of E.Coli and other health hazards  
28 have presented serious problems to the general population  
29 and have raised concerns regarding the safety of the food  
30 supply.

31 It is an objective of the present invention to  
32 provide a biological material detecting system for  
33 protecting the consumer by detecting and unmistakably  
34 presenting to the untrained eye visual icons on the  
35 packaging material which signify the presence of a number



1 of pathogens in the food stuff or other materials which  
2 are at a level harmful to humans.

3 It is another objective of the instant invention to  
4 provide a bioassay material wherein an antigen detecting  
5 antibody system is immobilized upon the surface of a  
6 flexible polyolefin film.

7 It is still another objective of the instant  
8 invention to provide a bioassay material wherein an  
9 antigen detecting antibody system is immobilized upon the  
10 surface of a flexible polyvinylchloride film.

11 It is a further objective of the invention to provide  
12 a biological material detecting system which is so similar  
13 in appearance and utilization that its use, in lieu of  
14 traditional packaging materials, is not apparent to the  
15 food processor or other packagers.

16 A still further objective of the present invention is  
17 to provide a biological material detecting system which is  
18 cost effective when compared to traditional packaging  
19 materials.

20 Other objectives and advantages of this invention  
21 will become apparent from the following description taken  
22 in conjunction with the accompanying drawings wherein are  
23 set forth, by way of illustration and example, certain  
24 embodiments of this invention. The drawings constitute a  
25 part of this specification and include exemplary  
26 embodiments of the present invention and illustrate  
27 various objects and features thereof.

28

#### 29 **Brief Description of the Figures**

30 Figure 1 is a cross-sectional interpretation of an  
31 antibody sandwich immunoassay device;

32 Figure 2 is a cross-sectional interpretation of a single  
33 ligand assay;

34 Figure 2A is a cross-sectional interpretation of a single  
35 ligand assay including a chromogenic ligand;



1 Figure 3 is a diagrammatic representation showing the  
2 functioning of a single ligand assay;  
3 Figure 4 is a cross-sectional interpretation of an  
4 antibody sandwich immunoassay including a scavenger system  
5 for microbial quantification;  
6 Figures 5 and 6 are a diagrammatic representation showing  
7 the functioning of a sandwich assay/scavenger system;  
8 Figure 7 is a planar view of an example of icon placement  
9 and printing;  
10 Figure 7A is an example of a typical code of  
11 identification applied to the icon pattern;  
12 Figure 8 is the result derived from EXAMPLE 2 and  
13 exemplifies capture sensitivity of a single ligand treated  
14 polyvinylchloride film;  
15 Figure 9 is a block diagram of the apparatus illustrating  
16 the process steps for forming a sandwich assay;  
17 Figure 10 is a block diagram of the apparatus illustrating  
18 the process steps for forming a single ligand assay.

19  
20 **Description of the Preferred Embodiment(s)**

21 Referring now to Figure 1, the detection and  
22 identification of various biological materials in packaged  
23 foods or other products is accomplished by the use of  
24 antibodies which are specific to the biological material  
25 being sought. Specific antibodies, defined as capture  
26 antibodies, are biologically active ligands characterized  
27 by their ability to recognize an epitope of the particular  
28 toxic substance being tested for. These capture  
29 antibodies are selected from such materials as antibodies,  
30 aptamers, single stranded nucleic acid probes, lipids,  
31 natural receptors, lectins, carbohydrates and proteins.  
32 In one embodiment of the invention, the capture antibodies  
33 are arranged with unique icon shapes and in particular  
34 patterns. The capture antibodies are immobilized to the  
35 polymer film. An agarose gel coat containing detector



1     antibodies is printed in register above the capture  
2     antibodies. A protective gel coat completes the  
3     construction of the packaging material. The gel coat  
4     constituting the inner layer, e.g. that layer which is  
5     next to the packaged product, is a special type of gel  
6     coat or an equivalent thereto which has sufficient  
7     porosity to allow toxic molecules, known as antigens, to  
8     migrate through it to an antibody "sandwich" laminated  
9     between the polymer film and the gel coat. The special  
10    gel coat has sufficient abrasion resistance to prevent  
11    exposure of the reagents to the product. The special gel  
12    coat useful in the invention is a readily available  
13    coating commonly utilized in the food industry to coat  
14    candies and the like, e.g. coated chocolates to prevent  
15    them from melting on one's hands. Migration of antigens  
16    is driven by capillary action and normally reaches a state  
17    of equilibrium within a 72 hour time period. In a  
18    particularly preferred embodiment, when operating within a  
19    temperature range of 4 - 25 degrees Celsius, an initial  
20    positive reading can be obtained within 30 minutes, and  
21    the test continues to yield results for about 72 hours.  
22    Upon migrating through the special gel coat the antigen  
23    enters an agarose gel film which has surfactant  
24    properties, contains free detector antibodies, and also  
25    contains one or more ingredients designed to enhance the  
26    growth of microbial materials, e.g. nutrients such as  
27    sorbitol, NOVOBIOCIN, CEFIXIME and TELLURITE which  
28    increase the growth rate and ease isolation of E. Coli  
29    0157H. If the antigen encounters a species of antibody  
30    which is specific to an epitope thereof, it will then bind  
31    to it forming a detector/antibody complex. Once bound  
32    thereto, the bound antigen/antibody complex becomes too  
33    large to migrate back through the special gel coat due to  
34    its inherent fine porous structure. This insures that  
35    pathogenic material can not migrate back into the product



1 being tested. Continuing pressure toward equilibrium from  
2 capillarity will tend to move the antigen, with its bound  
3 antibody, through a second gel coat layer and into an area  
4 of the flexible polyvinylchloride or polyolefin film  
5 containing corresponding species of immobilized capture  
6 antibodies. The layer of immobilized antibodies is  
7 attached to the outer polymer film in predetermined  
8 patterns of simple icons, as best seen in Figures 7, 7A.  
9 When the particular species of bound antigen encounters a  
10 particular corresponding species of immobilized antibody  
11 specific to a separate and distinct epitope thereof,  
12 further binding occurs. Upon the antigen binding to the  
13 two antibodies, a distinct icon shape emerges on the outer  
14 film at the point of binding, thereby providing a visual  
15 indicator.

16 While it is theoretically possible to detect an  
17 unlimited number of pathogens present in a packaged  
18 product, then to present this information in a very clear  
19 and unmistakable manner to an untrained consumer, as a  
20 practical matter there are limits to the amount of  
21 information which can be developed and presented in the  
22 biological material detecting system. Some of the  
23 limiting factors are cost, available surface area for  
24 display of information, complexity, and other  
25 considerations. Thus, for illustrative purposes only, the  
26 biological material detecting system as exemplified herein  
27 utilizes four separate pairs of antibodies, as set forth  
28 in Figures 7 and 7A. This is in no way meant to suggest a  
29 limit on the number of antibodies that can be utilized in  
30 a single biological material detecting system.

31 As demonstrated in Figures 7 and 7A, the invention is  
32 exemplified with reference to detection of the following  
33 four microbes:

- 34 1. E-Coli;
- 35 2. Salmonella;



1     3. Listeria; and

2     4. Cyclospora.

3           To each of the four microbes, a particular icon shape  
4     is assigned. Although there are infinite numbers of icons  
5     which might be used including letters, numbers, or even  
6     words, we have chosen simple identifiers for the purpose  
7     of demonstration. As an initial step in the construction  
8     of the biological material detecting system, the outer  
9     polymer film or base layer undergoes a printing process in  
10    which a pattern of the four icons, wherein each icon  
11    utilizes a specific species of immobilized capture  
12    antibody, is applied thereto. Corresponding species of  
13    free antibodies, known as detector antibodies, which are  
14    biologically active ligands characterized by their ability  
15    to recognize a different epitope of the same particular  
16    toxic substance being tested for, and suspended in an  
17    agarose gel solution containing a surfactant and a  
18    nutrient, are printed in registration with the immobilized  
19    antibodies so as to be in overlying and juxtaposed  
20    relationship thereto, and are then dried. Lastly, a  
21    second gel coat having a degree of porosity sufficient to  
22    prevent passage of the detector antibodies is laminated to  
23    the preparation.

24           Although the detection of biological materials  
25    through the use of antibodies is well known, there are  
26    several new and novel aspects to the application of  
27    antibody science which are set forth in the development of  
28    the biological material detecting system of the present  
29    invention.

30           Among these are: 1) the use of multiple antibodies to  
31    detect multiple biological materials in individual  
32    packages; 2) the use of a distinctive icon or other shape  
33    to not only detect, but visually identify the biological  
34    materials to the consumer, vendor, regulator, etc.;  
35    3) insuring that detection and identification of the



1 biological materials is accomplished in a timely manner in  
2 each particular application by judiciously controlling the  
3 porosity of the gel coat, thereby controlling the lapse  
4 rate of the reaction through the strength of capillary  
5 action; 4) inclusion of additives within the special gel  
6 coat to enhance the levels of microbes present; 5)  
7 incorporating the biological material detecting system of  
8 the instant invention within the existing packaging  
9 industry infrastructure; and 6) providing a bioassay  
10 material and methods for its production and use which  
11 immobilizes the antibodies onto the surface of a flexible  
12 polyvinylchloride or polyolefin, e.g. a polyethylene, a  
13 surface treated polyethylene, a polypropylene, a surface  
14 treated polypropylene or mixture thereof.

15 The embodiment discussed above is based upon a  
16 sandwich immunoassay as depicted in Figure 1, which  
17 measures specific microbes, wherein the particular toxic  
18 substance is one or more members selected from the group  
19 consisting of a particular microorganism, biological  
20 materials containing the genetic characteristics of said  
21 particular microorganism, and mutations thereof. In a  
22 particular embodiment, the toxic substance is selected  
23 from the group consisting of microorganisms, nucleic  
24 acids, proteins, integral components of microorganisms and  
25 combinations thereof.

26 It should also be understood that the invention will  
27 function by direct measurement of microbes with certain  
28 types of antibodies, selected from the group consisting of  
29 an antibody, a single stranded nucleic acid probe, an  
30 aptamer, a lipid, a natural receptor, a lectin, a  
31 carbohydrate and a protein. The biological materials may  
32 also be measured by non-immunological methods in  
33 particular using labeled molecules, such as aptamers,  
34 which have a high affinity for the biological materials.

35



1           The invention utilizes various types of detector  
2   antibodies, e.g. those conjugated with dyes to produce a  
3   visual cue, or alternatively, photoactive compounds  
4   capable of producing a visual cue in response to a  
5   particular type of light exposure, for example a scanning  
6   system which detects luminescent properties which are  
7   visualized upon binding of the antigen and antibody. In  
8   this method of construction biological materials are  
9   measured directly with a biologically active ligand, e.g.  
10   an antibody, aptamer, nucleic acid probe or the like,  
11   which induces a conformational change to produce a visual  
12   cue.

13           It is also understood that specific polymers may be  
14   incorporated into the invention and that when a biological  
15   material is bound to the surface it induces a molecular  
16   change in the polymer resulting in a distinctly colored  
17   icon. Referring to Figures 2 and 2A, in an alternative  
18   embodiment a sandwich-type of construction is not  
19   necessary. As depicted in Figures 2 and 2A, the provision  
20   of certain types of biologically active ligand, e.g.  
21   chromogenic ligands to which receptors are bound will  
22   permit the visual confirmation of binding of the antigen  
23   to the immobilized ligand.

24           As depicted in Figure 3, a polymer film is provided  
25   and a biologically active ligand, preferably a chromogenic  
26   ligand, is immobilized to the polymer film. In the past,  
27   immobilized ligands were attached to rigid solid support  
28   matrices such as plastic, polystyrene beads, microtitre  
29   plates, latex beads, fibers, metal and glass surfaces and  
30   the like. The immobilized ligands have also been attached  
31   to flexible surfaces such as nitrocellulose or polyester  
32   sheets which were not transparent. Surprisingly, the  
33   inventor has discovered that it is possible to attach  
34   biologically active ligands to the surface of various  
35   flexible polymeric films, for example polyvinylchloride



1 and polyolefins, e.g. a polyolefin sheet having  
2 appropriate properties of transparency and flexibility and  
3 that the composite functions as a biological sensor or  
4 assay material. After printing on the polymer film, the  
5 material goes through a drying step; subsequent to which a  
6 special gel coat or liquid film is applied as a protectant  
7 layer and the final product is then dried.

8 Illustrative of films which will function in the  
9 present invention is a film containing a structural  
10 polymer base having a treated surface and incorporating  
11 therein a fluorescing antibody receptor and finally a  
12 stabilized gel coat. These films may be untreated  
13 polyethylene or polyvinylchloride films which are amenable  
14 to antibody immobilization by various mechanisms, e.g. by  
15 adsorption. In a particular embodiment, the films may be  
16 first cleaned, e.g. by ultrasonication in an appropriate  
17 solvent, and subsequently dried. For example the polymer  
18 sheet may be exposed to a fifteen minute ultrasonic  
19 treatment in a solvent such as methylene chloride,  
20 acetone, distilled water, or the like. In some cases, a  
21 series of solvent treatments are performed. Subsequently  
22 the film is placed in a desiccating device and dried.  
23 Alternatively, these films may be created by first  
24 exposing the film to an electron discharge treatment at  
25 the surface thereof, then printing with a fluorescing  
26 antibody receptor. Subsequently, a drying or heating step  
27 may be utilized to treat the film to immobilize the  
28 receptor. Next, the film is washed to remove un-  
29 immobilized receptor; the film is then coated with a gel  
30 and finally dried.

31 Additional modifications to polyolefin films may be  
32 conducted to create the presence of functional groups, for  
33 example a polyethylene sheet may be halogenated by a free  
34 radical substitution mechanism, e.g. bromination,  
35 chlorosulfonation,, chlorophosphorylation or the like.



1 Furthermore, a halodialkylammonium salt in a sulfuric acid  
2 solution may be useful as a halogenating agent when  
3 enhanced surface selectivity is desirable.

4 Grafting techniques are also contemplated wherein  
5 hydrogen abstraction by transient free radicals or free  
6 radical equivalents generated in the vapor or gas phase is  
7 conducted. Grafting by various alternative means such as  
8 irradiation, various means of surface modification,  
9 polyolefin oxidation, acid etching, inclusion of chemical  
10 additive compounds to the polymer formulation which have  
11 the ability to modify the surface characteristics thereof,  
12 or equivalent techniques are all contemplated by this  
13 invention.

14 Additionally, the formation of oxygenated surface  
15 groups such as hydroxyl, carbonyl and carboxyl groups via  
16 a flame treatment surface modification technique is  
17 contemplated.

18 Further, functionalization without chain scission by  
19 carbene insertion chemistry is also contemplated as a  
20 means of polyolefin polymer modification.

21 Illustrative of the types of commercially available  
22 films which might be utilized are polyvinylchloride films  
23 and a straight polyethylene film with electron discharge  
24 treatment marketed under the trademark SCLAIR®. The  
25 electron discharge treatment, when utilized, renders the  
26 film much more susceptible to immobilization of the  
27 antibodies on its surface. Additional films which might  
28 be utilized are Nylon 66 films, for example DARTEK®, a  
29 coextrudable adhesive film such as BYNEL® and a blend of  
30 BYNEL® with polyethylene film.

31 With reference to Figures 4-6, one of the most  
32 important features of the biological material detecting  
33 system is its ability to quantitatively sensitize the  
34 antibody or aptamer so as to visually identify only those  
35 biological materials that have reached a concentration



1 level deemed harmful to humans. One means of providing  
2 this sensitization is by including a scavenger antibody  
3 which is a biologically active ligand characterized as  
4 having a higher affinity for the particular toxic  
5 substance than the capture antibody. The scavenger  
6 antibody is provided in a sufficient amount to bind with  
7 the particular toxic substance up to and including a  
8 specific threshold concentration. In this manner, the  
9 capture antibody will be prevented from binding with a  
10 detector antibody until the concentration of the  
11 particular biological material surpasses the specific  
12 threshold concentration. In this manner, the biological  
13 material detecting system visually reports only those  
14 instances where concentration levels are deemed harmful by  
15 health regulatory bodies.

16 Since the biological material detecting system as  
17 described herein can maintain its activity over long  
18 periods of time, e.g. up to 1 year, it is able to protect  
19 against contamination in products which have long shelf  
20 lives. Additionally, by reporting only toxic  
21 concentrations, it avoids "false positives" and, in some  
22 cases, can extend the useful life of the product.

23 Referring to Figures 9 and 10, the apparatus for  
24 producing the biological material detecting system is  
25 illustrated. These embodiments are essentially particular  
26 combinations of printers, coaters and dryers which will be  
27 used to place biologically active reagents upon a thin  
28 polymer film useful for packaging food stuffs and other  
29 products. These films will be further processed  
30 subsequent to application of the biological material  
31 detecting system by printing, laminating, or equivalent  
32 methods of fabrication. The machinery is designed so that  
33 it will transport and process very thin films at rather  
34 high speeds. Furthermore, the machinery is designed so  
35 that it can be utilized effectively as an additional



1 processing step when added to continuous processing  
2 operations already in use at packaging material  
3 fabrication plants. The printing machinery is designed so  
4 that a minimum of four distinct biological active ligands  
5 in a hydrate solution can be printed in patterns in a  
6 precise registration on the polymer film. The printing  
7 may be accomplished by jet spray or roller application, or  
8 equivalent printing methods. Each print applicator is  
9 capable of printing a detailed icon no larger than 1/4" x  
10 1/4" in a minimum thickness. Patterning may be controlled  
11 by computer or roller calendaring. It is important to  
12 determine the appropriate viscosity of the solution to be  
13 applied so that successful printing, coating, and drying  
14 can be accomplished. After the printing step the icons  
15 must be protected. This is accomplished by a final  
16 application of a thin special gel coat or a thin liquid  
17 film. This step is accomplished by a 100% coating of the  
18 entire film or alternatively by selectively coating each  
19 icon such that a 10% overlap is coated beyond the icon in  
20 all directions. This coating step may be accomplished  
21 with sprays or rollers and the viscosity of the coating  
22 material must be optimized so as to provide adequate  
23 coverage. The biological material detecting system must  
24 be dried after printing and once again after coating. The  
25 drying is accomplished in a very rapid manner so as to  
26 enable high through put for the process. Various means of  
27 drying include the use of radiant heat, convected air and  
28 freeze drying. Care must be taken to avoid drying  
29 temperatures which will inactivate the biological reagents  
30 which have been applied. The polymer film which has been  
31 surface treated in the form of electron discharge, e.g.  
32 corona treatment, is most preferred. After preparation,  
33 the thin film is transported at relatively high speeds so  
34 that a wrinkle free surface is provided for printing,  
35 coating and rollup. Additionally, the apparatus provides



1 a complete recovery system for the reagents which allows  
2 for total recovery of the agents and the volatile organic  
3 contaminants.

4 The invention will be further illustrated by way of  
5 the following examples:

6 **EXAMPLE 1**

7 **Detection of Antibody on the Surface of a Thin Layer**  
8 **Polyvinylchloride Sheet:**

9 Rabbit polyclonal IgG was diluted to a final concentration  
10 of 2.0 µg/ml in 0.1M carbonate (Na<sub>2</sub>CO<sub>3</sub>)-bicarbonate  
11 (NaHCO<sub>3</sub>) buffer, pH 9.6.

12 Using a 2" x 3" grid, 75 µL (150 ng) was applied to a  
13 sheet of polyvinylchloride at 1" intervals.

14 The antibody treated polyvinylchloride sheet was dried for  
15 1.5 hrs. at a temperature of 37°C.

16 The dried sheet was then washed 3 times with a phosphate  
17 buffered saline solution at a pH of 7.4.

18 HRP conjugated goat anti-rabbit IgG (G<sub>α</sub>R<sup>HRP</sup>) was diluted to  
19 a concentration of 1:7000 in 1% casein, 0.1M potassium  
20 ferricyanide K<sub>3</sub>Fe(CN)<sub>6</sub>, 0.1% phosphate glass (Na<sub>15</sub>P<sub>13</sub>O<sub>40</sub> -  
21 Na<sub>20</sub>P<sub>18</sub>O<sub>55</sub>), at a pH of 7.4.

22 A precision pipette was used to apply 125 µL of diluted  
23 G<sup>HRP</sup> to the grid backed polyvinylchloride sheet at 1"  
24 intervals coinciding with the area covered by the  
25 previously coupled R<sub>α</sub>G.

26 The sheet was incubated at room temperature for 30  
27 minutes.

28 The sheet was then washed 3X with phosphate buffered  
29 saline at a pH of 7.4.

30 125µL of precipitating TMB enzyme substrate was added to  
31 the test areas.

32 The sheet was incubated at room temperature until color  
33 development was complete.

34 Lastly the sheet was washed 3 times with deionized water  
35 and allowed to air dry.



**EXAMPLE 2****Full Sandwich Immunoassay on the Surface of a Thin Layer Polyvinylchloride Sheet**

Rabbit polyclonal IgG was diluted to a final concentration of 2.0 µg/ml in 0.1M carbonate ( $\text{Na}_2\text{CO}_3$ )-bicarbonate ( $\text{NaHCO}_3$ ) buffer, pH 9.6.

A 13 x 9 cm piece of thin layered polyvinylchloride sheet was inserted into a BIO-RAD DOT-SPOT apparatus possessing 96 sample wells spaced at 1.0 cm intervals in a 12 x 8 well grid.

A 100 µL sample (1.0 µg) of rabbit polyclonal IgG was applied to each well 8 of column 1.

Antibody samples applied to columns 2-12 represented serial dilutions of the antibody ranging from 500 ng - 0.5 ng.

The antibody treated polyvinylchloride sheet was dried overnight at 37° C.

The dried sheet was washed 3 times with phosphate buffered saline (PBS), pH 7.4.

Antigen was diluted to a final concentration of 1.0 µg/ml in tris buffered saline (TBS) with 1% casein, pH 7.4.

100 µL, representing 100 ng, of antigen, was applied to each well of the apparatus and incubated at room temperature for 1 hour.

The polyvinylchloride sheet was washed 3 times with phosphate buffered saline (PBS), pH 7.4.

Detector mouse monoclonal antibody was diluted was diluted 1:625 with TBS containing 1% casein, 0.1M potassium ferricyanide  $\text{K}_3\text{Fe}(\text{CN})_6$ , and 0.1% phosphate glass ( $\text{Na}_{15}\text{P}_{13}\text{O}_{40}$  -  $\text{Na}_{20}\text{P}_{18}\text{O}_{55}$ ), pH 7.4.

100 µL of the 1:625 dilution of detector antibody solution was applied to each well of row # 1.

Detector samples of 100 µL applied to rows 2-7



1 represented serial dilutions of the antibody ranging from  
2 1:1,250 to 1:80,000. Dilutions of detector antibody were  
3 incubated on the polyvinylchloride sheet for 1 Hr. at room  
4 temperature.

5 The polyvinylchloride sheet was washed 3 times with  
6 phosphate buffered saline (PBS), pH 7.4.

7 100 µL of goat anti-mouse IgG<sup>HRP</sup> were added to each  
8 well of the DOT-SPOT apparatus and allowed to incubate for  
9 one hour at room temperature.

10 The polyvinylchloride sheet was washed 3 times with  
11 phosphate buffered saline (PBS), pH 7.4.

12 100 µL of precipitating TMB enzyme substrate was  
13 added to the test areas.

14 The sheet was incubated at room temperature until  
15 color development was complete (see Figure 8).

16 Lastly the sheet was washed 3 times with deionized  
17 water and allowed to air dry.

18 It is to be understood that while a certain form of  
19 the invention is illustrated, it is not to be limited to  
20 the specific form or arrangement of parts herein described  
21 and shown. It will be apparent to those skilled in the  
22 art that various changes may be made without departing  
23 from the scope of the invention and the invention is not  
24 to be considered limited to what is shown in the drawings  
25 and described in the specification.

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# CLAIMS

What is claimed is:

Claim 1. A biological assay material for detecting the presence of a particular toxic substance comprising:  
a base layer which is a flexible film for immobilization of a ligand applied to a surface thereof and wherein said film is selected from the group consisting of a polyolefin or a polyvinylchloride;  
a capture antibody which is a biologically active ligand characterized by its ability to recognize an epitope of the particular toxic substance, said ligand being immobilized onto said surface of said film;  
a first agarose gelcoat layer overlying the capture antibody, said agarose layer being permeable to the toxic substance and containing at least one ingredient to enhance the growth thereof, said layer further containing a detector antibody which is a biologically active ligand characterized by its ability to recognize a different epitope of said particular toxic substance, thereby forming a detector antibody/antigen complex; and  
a second protective gelcoat layer overlying the detector antibody and having a degree of porosity whereby passage of said toxic substance is permitted and passage of said detector antibody/antigen complex is prevented, said second protective gelcoat layer having a degree of abrasion resistance effective to protect the biological assay material.



1           Claim 2. The biological assay material according to  
2           claim 1 wherein the flexible film is a polyolefin selected  
3           from the group consisting of polyethylene, polypropylene  
4           and mixtures thereof.

5

6           Claim 3. The biological assay material according to  
7           claim 1 wherein the flexible film is a polyvinylchloride.

8

9           Claim 4. The biological assay material according to  
10          claim 1 wherein the particular toxic substance is one or  
11          more members selected from the group consisting of a  
12          particular microorganism, biological materials containing  
13          the genetic characteristics of said particular  
14          microorganism, and mutations thereof.

15

16          Claim 5. The biological assay of material according  
17          to claim 1 wherein the particular toxic substance is  
18          selected from the group consisting of microorganisms,  
19          nucleic acids, proteins, integral components of  
20          microorganisms and combinations thereof.

21

22          Claim 6. The biological assay material according to  
23          claim 1 wherein the ligand is selected from the group  
24          consisting of an antibody, a single stranded nucleic acid  
25          probe, an aptamer, a lipid, a natural receptor, a lectin,  
26          a carbohydrate and a protein.

27

28          Claim 7. The biological assay material according to  
29          claim 1 further including a scavenger antibody which is a  
30          biologically active ligand characterized as having a  
31          higher affinity for the particular toxic substance than  
32          the capture antibody, said scavenger antibody being  
33          present in a sufficient amount to bind with the particular  
34          toxic substance up to and including a specific threshold  
35          concentration;



1           whereby a capture antibody will be prevented from  
2           binding with a detector antibody until the concentration  
3           of the particular biological material surpasses the  
4           specific threshold concentration.

5

6           Claim 8. A method to detect the presence or absence  
7           of a particular toxic substance, which method comprises:

8           a) providing a base layer which is a flexible film  
9           for immobilization of a ligand applied to a surface  
10          thereof and wherein said film is selected from the group  
11          consisting of a polyolefin or a polyvinylchloride;

12          b) providing a capture antibody which is a  
13          biologically active ligand characterized by its ability to  
14          recognize an epitope of the particular toxic substance,  
15          said ligand being immobilized onto said surface of said  
16          flexible film;

17          c) providing a first agarose gelcoat layer overlying  
18          the capture antibody, said agarose layer being permeable  
19          to the toxic substance and containing at least one  
20          ingredient to enhance the growth of the toxic substance,  
21          said layer further containing a detector antibody which is  
22          a biologically active ligand characterized by its ability  
23          to recognize a different epitope of said particular toxic  
24          substance;

25          d) providing a second protective gelcoat layer  
26          overlying the detector antibody and having a degree of  
27          porosity sufficient to prevent passage of said detector  
28          antibody therethrough;

29          e) placing said biological assay material in an  
30          environment which may contain a particular toxic  
31          substance; and

32          f) monitoring said biological assay material for a  
33          period of time sufficient to observe a visual signal which  
34          will confirm the presence or absence of the particular  
35          toxic substance.



1

2           Claim 9. A material useful for food packaging and  
3 characterized by its ability to detect the presence and  
4 particularly identify one or more toxic substances  
5 comprising:

6           a base layer which is a flexible film for  
7 immobilization of a ligand applied to a surface thereof  
8 and wherein said film is selected from the group  
9 consisting of a polyolefin or a polyvinylchloride;

10          a capture antibody which is a biologically active  
11 ligand characterized by its ability to recognize an  
12 epitope of the particular toxic substance, said ligand  
13 being immobilized onto said surface of said flexible film;

14          a first protective agarose gelcoat layer overlying  
15 the capture antibody, said agarose layer being permeable  
16 to the toxic substance;

17          a detector antibody which is a biologically active  
18 ligand characterized by its ability to recognize a  
19 different epitope of said particular toxic substance, said  
20 detector antibody overlying said first protective gelcoat  
21 layer and in juxtaposed relation thereto; and

22          a second gelcoat layer overlying the detector  
23 antibody and having a degree of porosity sufficient to  
24 prevent passage of said detector antibody therethrough.

25

26           Claim 10. The material according to claim 9 wherein  
27 the flexible polyolefin film is selected from the group  
28 consisting of polyethylene, polypropylene and mixtures  
29 thereof.

30

31           Claim 11. The material according to claim 9 wherein  
32 the flexible film is a polyvinylchloride.

33

34           Claim 12. The material according to claim 9 wherein  
35 the particular toxic substance is one or more members



1 selected from the group consisting of a particular  
2 microorganism, biological materials containing the genetic  
3 characteristics of said particular microorganism, and  
4 mutations thereof.

5  
6 Claim 13. The material according to claim 9 wherein  
7 the particular toxic substance is selected from the group  
8 consisting of microorganisms, nucleic acids, proteins,  
9 integral components of microorganisms and combinations  
10 thereof.

11  
12 Claim 14. The material according to claim 9 wherein  
13 the ligand is selected from the group consisting of an  
14 antibody, a single stranded nucleic acid probe, an  
15 aptamer, a lipid, a natural receptor, a lectin, a  
16 carbohydrate and a protein.

17  
18 Claim 15. The material according to claim 9 further  
19 including a scavenger antibody which is a biologically  
20 active ligand characterized as having a higher affinity  
21 for the particular toxic substance than the capture  
22 antibody, said scavenger antibody being present in a  
23 sufficient amount to bind with the particular toxic  
24 substance up to and including a specific threshold  
25 concentration;

26 whereby a capture antibody will be prevented from  
27 binding with a detector antibody until the concentration  
28 of the particular biological material surpasses the  
29 specific threshold concentration.

30  
31 Claim 16. The material according to claim 9 wherein  
32 one or more species of capture antibody are  
33 immobilized onto said surface of said flexible film in a  
34 particular orientation, each of said one or more species  
35 being characterized by a unique shape; and



1           one or more corresponding species of detector  
2   antibody are applied onto the surface of said first  
3   protective gelcoat layer in the same particular  
4   orientation as said one or more species of capture  
5   antibody, each of said one or more species being  
6   characterized by a corresponding unique shape;

7           whereby simultaneous binding of any of the one or  
8   more species of capture antibodies and one or more  
9   corresponding species of detector antibodies with the  
10   particular toxic substance which they recognize results in  
11   the appearance of a visual signal having the unique shape  
12   assigned to that species;

13          wherein an observer is alerted to the presence and  
14   identity of said particular toxic substance.

15

16          Claim 17. A biological assay material for detecting  
17   the presence of a particular toxic substance comprising:

18          a base layer which is a flexible film for  
19   immobilization of a ligand applied to a surface thereof  
20   and wherein said film is selected from the group  
21   consisting of a polyolefin or a polyvinylchloride;

22          a biologically active ligand immobilized to the  
23   flexible film; and

24          a gel coat or liquid film applied as a protectant  
25   layer;

26          whereby binding of the particular toxic substance and  
27   biologically active ligand produces a visual signal which  
28   is indicative of both the presence and identity of said  
29   particular toxic substance.

30

31          Claim 18. The biological assay material according to  
32   claim 17 wherein the biologically active ligand is a  
33   chromogenic ligand.

34

35



1           Claim 19. The biological assay material according to  
2           claim 17 wherein the base layer is a film incorporating  
3           thereon a fluorescing antibody receptor.  
4

5           Claim 20. The biological assay material according to  
6           claim 19 wherein the base layer is created by printing  
7           with a fluorescing antibody receptor and drying or heating  
8           the film to immobilize said receptor.  
9

10          Claim 21. The biological assay material according to  
11          claim 17 wherein a scavenger antibody which is a  
12          biologically active ligand characterized as having a  
13          higher affinity for the particular toxic substance than  
14          the immobilized ligand is provided in a sufficient amount  
15          to bind with the particular toxic substance up to and  
16          including a specific threshold concentration;  
17          whereby the assay material is quantitatively  
18          sensitized so as to visually identify only those  
19          particular toxic substances that have reached a  
20          concentration level deemed harmful to humans.  
21

22          Claim 22. The biological assay material according to  
23          claim 18 wherein the chromogenic ligand is selected from  
24          the group consisting of those conjugated with dyes to  
25          produce a visual cue and those characterized as  
26          photoactive compounds capable of producing a visual cue in  
27          response to a particular type of light exposure;  
28          whereby binding of the particular toxic substance and  
29          chromogenic ligand results in a color change or  
30          visualization of a luminescent property which is  
31          indicative of both the presence and identity of said  
32          particular toxic substance.  
33  
34



1           Claim 23. The biological assay material according to  
2           claim 17 wherein the material is a food packaging  
3           material.

4

5           Claim 24. The biological assay material according to  
6           claim 17 containing a plurality of biologically active  
7           ligands, each of said ligands being receptive to an  
8           epitope of a different particular toxic substance and  
9           having a unique shape;

10          whereby upon binding with one or more of said  
11          different particular toxic substances, a visual signal  
12          will result thereby alerting an observer to the presence  
13          and identity of any or all of the particular toxic  
14          substances to which said material is receptive.

15

16          Claim 25. The biological assay material according to  
17          claim 17 wherein the particular toxic substance is one or  
18          more members selected from the group consisting of a  
19          particular microorganism, biological materials containing  
20          the genetic characteristics of said particular  
21          microorganism, and mutations thereof.

22

23          Claim 26. The biological assay of material according  
24          to claim 17 wherein the particular toxic substance is  
25          selected from the group consisting of microorganisms,  
26          nucleic acids, proteins, integral components of  
27          microorganisms and combinations thereof.

28

29          Claim 27. The biological assay material according to  
30          claim 17 wherein the ligand is selected from the group  
31          consisting of an antibody, a single stranded nucleic acid  
32          probe, an aptamer, a lipid, a natural receptor, a lectin,  
33          a carbohydrate and a protein.

34

35



1           Claim 28. The material according to claim 17 wherein  
2           the flexible polyolefin film is selected from the group  
3           consisting of polyethylene, polypropylene and mixtures  
4           thereof.

5

6           Claim 29. The material according to claim 17 wherein  
7           the flexible film is a polyvinylchloride.

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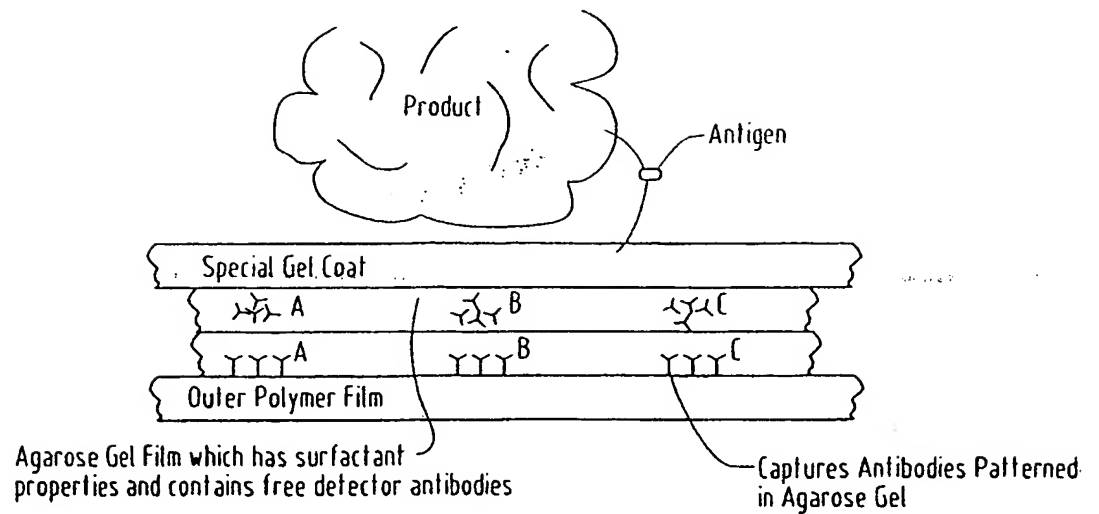
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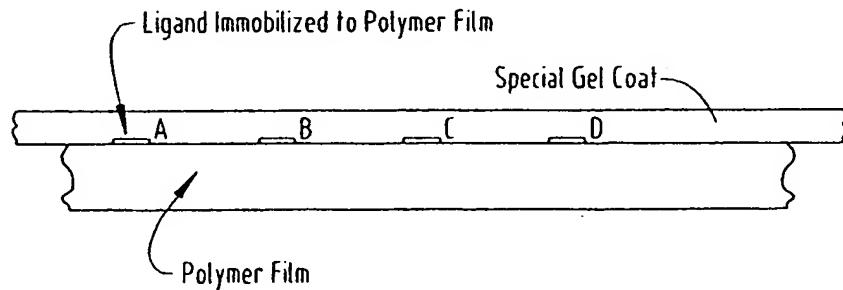
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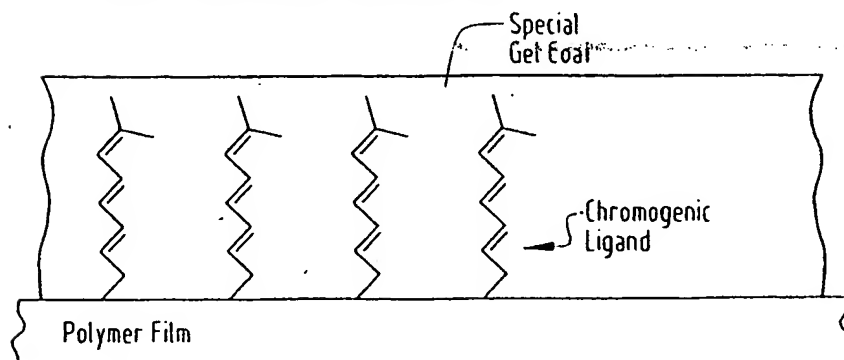


*FIG. 1*

Note: the approximate thickness of the antibody sandwich is 100 microns

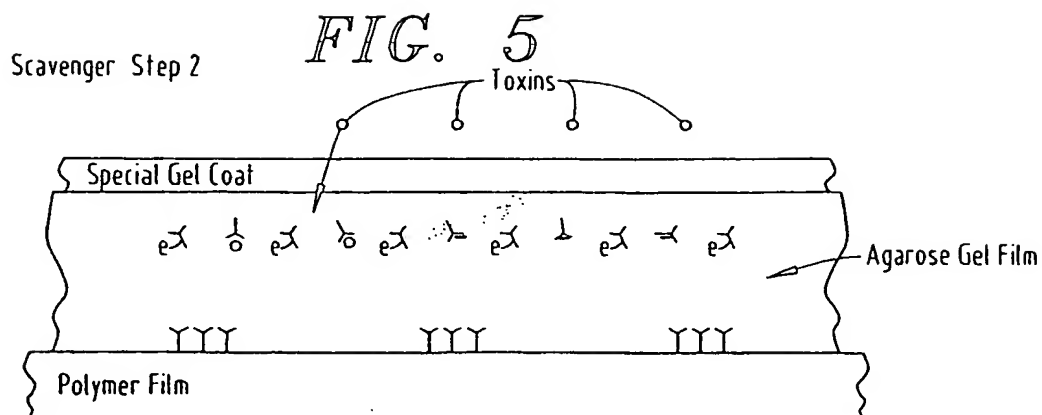
*FIG. 2**FIG. 2A*

## SINGLE LIGAND ASSAY CONSTRUCTION



A chromogenic ligand is immobilized on the polymer film in patterns of icons, and is coated with a porous gel which will allow the migration of toxins to the ligand.



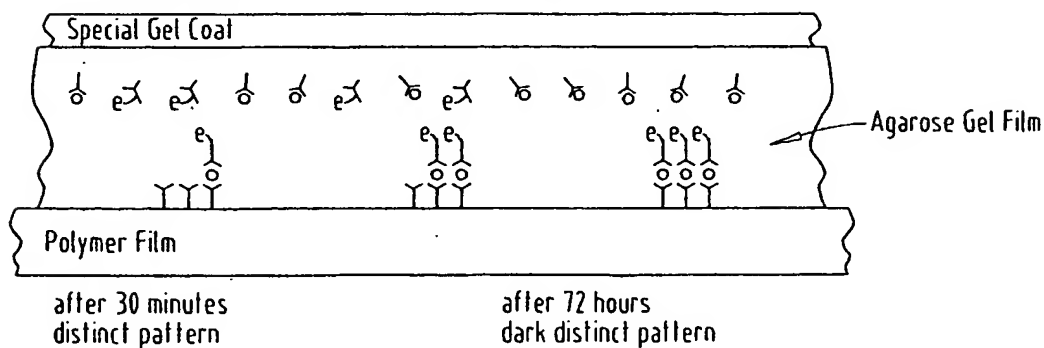


When toxins enter the sandwich, they will bind first with the unlabeled detector antibodies until all of these are bound.

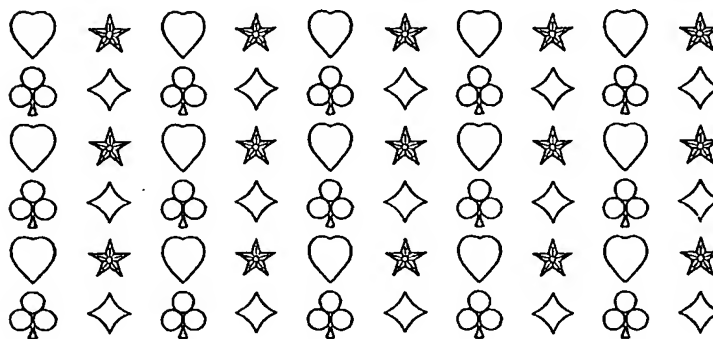
*FIG. 6*

Scavenger Step 3

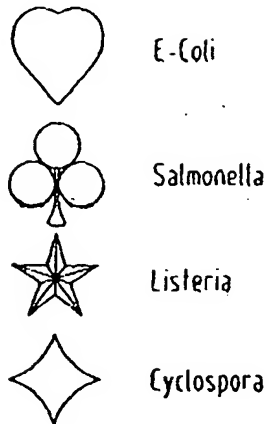
After all of the high affinity unlabeled detector antibodies are bound to the toxins, the detector antibodies labeled with a colorimetric enzyme will begin to bind to the toxins. The labeled complex will then begin to bind to the capture antibodies, producing a visual cue.



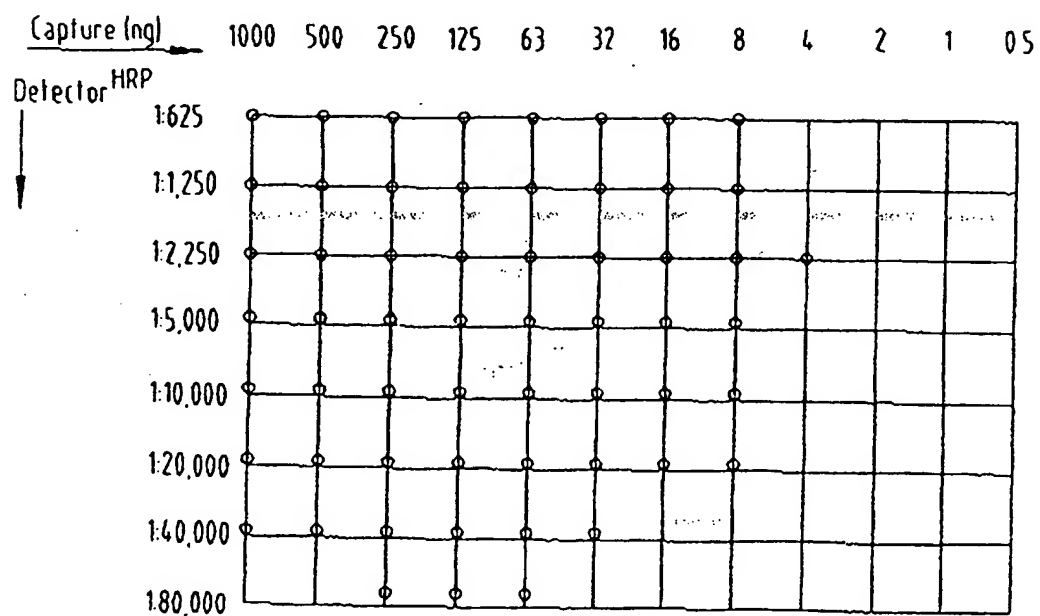
*FIG. 7*



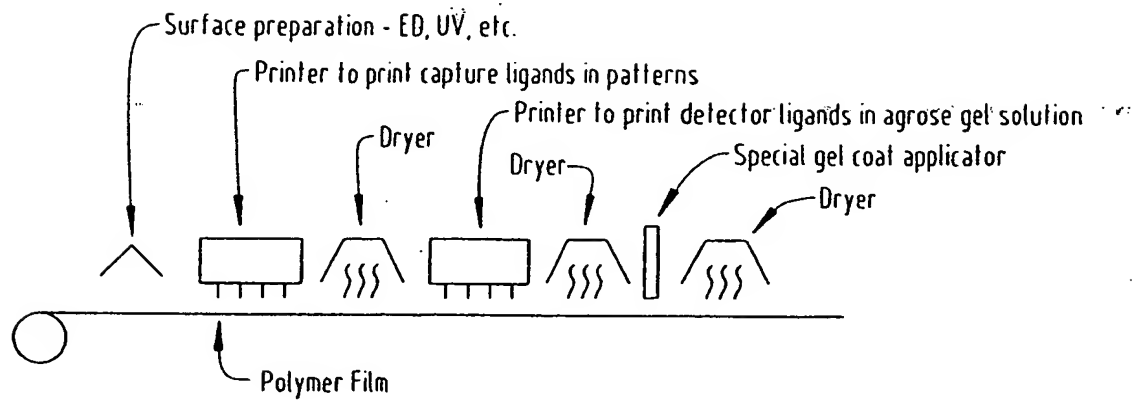


*FIG. 7A**FIG. 8*

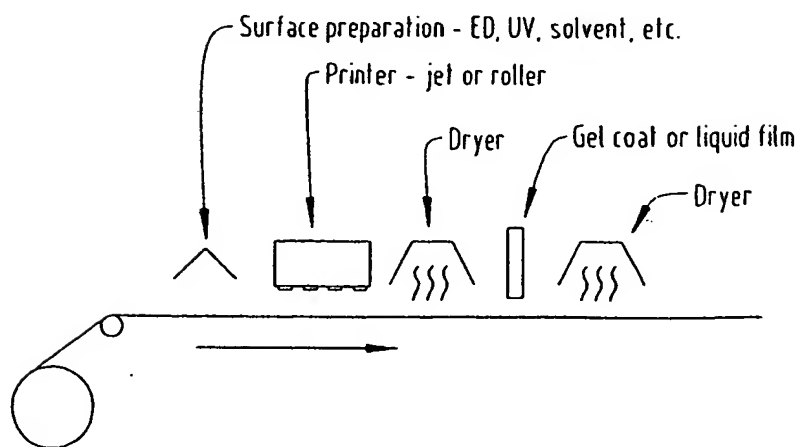
Checkerboard Dot-Spot Application of RaMBP on a Polyvinylchloride Surface and Detection by GaR<sup>HRP</sup>





*FIG. 9**FIG. 10*

## GENERAL LAYOUT APPLICATION MACHINERY





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(54) Title: METHOD AND APPARATUS FOR SELECTIVE BIOLOGICAL MATERIAL DETECTION

(57) Abstract: The present invention relates to bioassay materials useful for the detection of toxic substances and, more particu-  
larly, to packaging materials for food and other products, along with methods for their manufacture and use. The invention provides a  
unique composite material capable of detecting and identifying multiple biological material within a single package. The biological  
material identification system is designed for incorporation into existing types of flexible packaging material such as polyvinylchlo-  
ride or polyolefin films, and its introduction into the existing packaging infrastructure will require little or no change to present  
systems and procedures.



## A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

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X	EP 0 347 839 A (FUJIREBIO KK ;FUJI PHOTO FILM CO LTD (JP)) 27 December 1989 (1989-12-27)	17-29
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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